METLA
Metsäntutkimuslaitos
Skogsforskningsinstitutet
Finnish Forest Research Institute

# Cryopreservation of forest trees – potentials and applications in Metla

Leena Ryynänen and Tuija Aronen

## Introduction

Research on cryopreservation techniques was initiated for germplasm conservation and research purposes. In Metla, cryopreservation methods have been developed for the economically important forest trees in Finland, e.g. for silver birch (*Betula pendula*, Roth), hybrid aspen (*Populus tremula* L. x *Populus tremuloide*s Michx.) and Scots pine (*Pinus sylvestris* L.).

## Cryopreservation of birch and aspen

*Cryopreservation of in vivo material* using dormant vegetative buds is possible by slow cooling without any cryoprotectants. The recovery (58 - 92%) of buds after cryostorage is equal to initiation of micropropagation from non-cryopreserved buds.

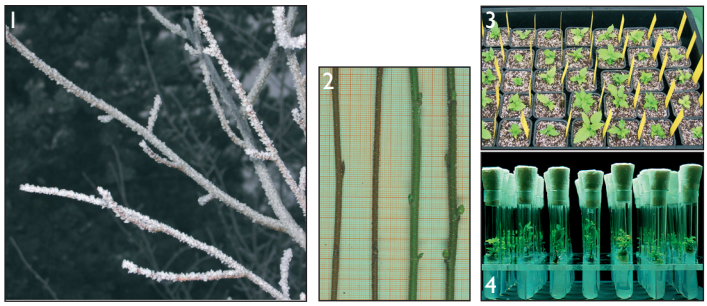


Fig. 1. Hybrid aspen at the time of outdoor collection. Fig. 2. The 2-year-old (on left) and 1-year-old (on right) birch plants cold hardened in the greenhouse. Fig. 3. Regenerated aspen plants. Fig. 4. Regeneration of silver birch from explants cold hardened in the greenhouse.

*Cryopreservation of in vitro material* of silver birch succeeds using slow cooling method and vitrification after cold hardening with ammonium substitution. That of aspen is successful only using vitrification. Cryoprotection in slow cooling method is performed by PGD and in vitrification by PVS2. The recovery of silver birch after vitrification (71%) was better than that after slow cooling method (52%). Recovery of vitrified hybrid aspen genotypes varied from 2.5% to 75%, being lower than the controls.

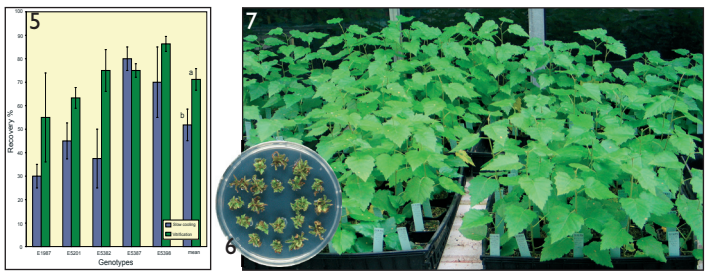


Fig.5. Recovery of different silver birch genotypes using slow cooling method and vitrification. Fig. 6. Recovery of silver birch cryopreserved by slow cooling. Fig. 7. Birches regenerated after vitrification.

Finnish Forest Research Institute, Punkaharju Research Unit, Finlandiantie 18,
FI-58450 Punkaharju, FINLAND
first name.last name@metla.fi

## Cryopreservation of Scots pine SE cultures

The best regrowth was achieved by using slow cooling method with PGD as cryopretectant. 78% of the cryopreserved lines remained viable after cryopreservation. After 7 weeks' cultivation the growth rates (Wo/Wi) of the lines varied from 3 to 24 being better than the untreated controls.

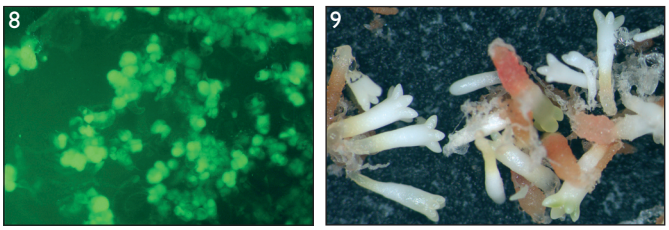


Fig. 8. Scots pine embryogenic culture stained with FDA after thawing.
Fig. 9. Maturating somatic embryos in SE culture following cryostorage.

## Genetic fidelity of the cryopreserved materials

For all the applications, genetic fidelity of cryopreserved materials is essential. In our study, growth rate and morphology of the regenerated plants after cryostorage were similar to controls in the greenhouse. In addition, the regenerated plants showed no genetic changes compared to the original donor trees.

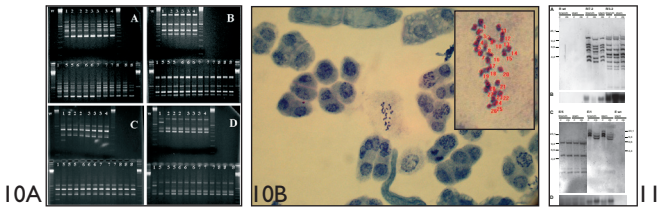


Fig. 10 A and 10 B. Regenerated silver birches evaluated using RAPD assays together with chromosome analysis. Fig 11. Cryostored transgenic silver birch showing transgene stability and functioning.

## Future prospects

The future applications include the following: I) Selected birch genotypes cryopreserved for breeding. II) Extensive cryostorage of specific forms of different tree species for gene conservation and landscaping purposes. III) Cryopreservation of coniferous SE-cultures during the field testing of clonal material for production of forest regeneration material.


Fig. 12. Decorative silver birch genotype cryopreserved for landscaping.
Fig. 13. Somatic embryo plants of Scots pine in the greenhouse ready for field test.

Metla/3389/EMat/02.2008. Photos: T. Aronen, S. Kontunen-Soppela, J. Lehto, L. Ryynänen and T. Nikkanen